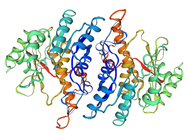


Cloning the *SOS4* gene

Noah Kirtley and Elizabeth Rueschhoff
Department of Biology, Indiana University Southeast, New Albany, IN 47150

Abstract

The *SOS4* (Salt overly sensitive 4) gene encodes a pyridoxal kinase that is involved in the biosynthesis of pyridoxal-5-phosphate, an active form of vitamin B6. Vitamin B6 plays an important role in cell metabolism. We utilized Polymerase Chain Reaction (PCR) to amplify the *SOS4* from cDNA. This product was then ligated into pGBKT7, a vector used in a Yeast 2-Hybrid protein interaction system, and transformed into *Escherichia coli.* After screening this construct for mutations, we will transform the construct into *Saccharomyces cerevisiae* and perform a Yeast 2-Hybrid screen, in which we will look for proteins that interact with SOS4.

Background Information

Vitamin B6 is a group of chemically similar compounds essential for many aspects of cell metabolism. The active form of Vitamin B6, Pyridoxal 5'-phosphate (PLP), serves as a cofactor in more than 140 biochemical reactions in cells (Gonzalez, 2007). These reactions are primarily related to amino acid biosynthesis and catabolism.

The *SOS4* gene plays an important role in the salvage pathways between different forms of vitamin B6. *SOS4* encodes for pyridoxal kinase (PL Kinase), the enzyme responsible for the conversion from pyridoxal to pyridoxal 5'-phosphate.

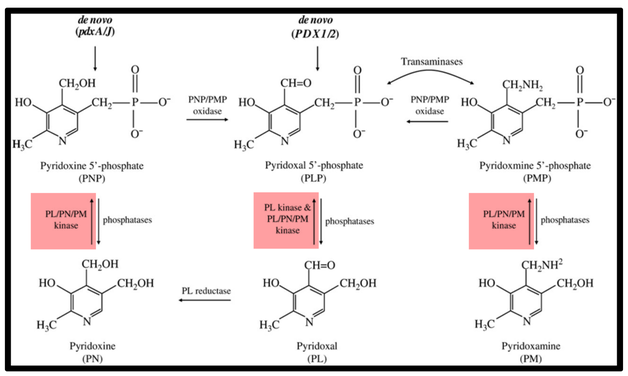


Figure 1. Vitamin B6 Salvage Pathways. Gonzalez et al, 2007.
The various ways in which different forms of Vitamin B6 are interconverted. The conversions in which pyridoxal kinase (PL Kinase, *SOS4*) is directly involved are highlighted in with red.

Prior to the beginning of our research, RNA was extracted from the leaf and flower of *Arabidopsis thaliana*. Complementary DNA (cDNA) was then synthesized using the RNA samples.

Methodology

1. PCR was performed to clone the gene from cDNA. Primers used were *SOS4* F 5' CATGGAGGCCGAATCCATGACGACGCCTCCAGTTCTA-TCT 3' and *SOS4* R 5' GCAGGTCGACGGATCCTCAGCTGTATCTTTCAGCTTTCAG 3'. Primestar MAX DNA Polymerase Mastermix (Takara) was utilized to clone the gene. (See Figure 3)
2. The cloned gene was ligated into the pGBKT7 vector (Figure 2) using the Infusion HD Cloning Kit (Clontech)
3. pGBKT7 containing the putative *SOS4* gene was transformed into *E. coli* (Stellar competent cells, Clontech) (Figure 4)
4. Colony PCR was performed to confirm the presence of *SOS4* in the transformed *E. coli* colonies. (Figure 5)
5. Colonies (Labeled 1-5) positive for the *SOS4* gene insert were grown in liquid culture.
6. The pGBKT7/*SOS4* plasmid construct was extracted from the transformed *E. coli* cells using the Wizard Plus SV Minipreps DNA Purification System (Promega).
7. DNA was quantified using Nanodrop and sent to sequencing.

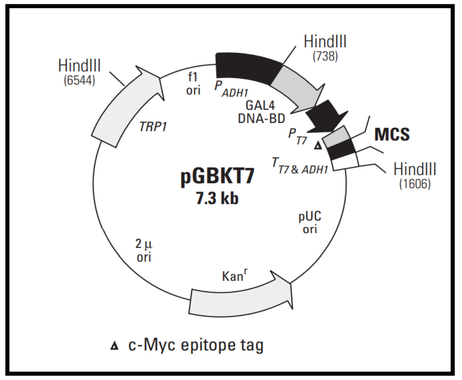


Figure 2. Restriction map of pGBKT7. Clontech, 2008.
The pGBKT7 vector utilized in transformation of *SOS4* into *E. coli*.

Results

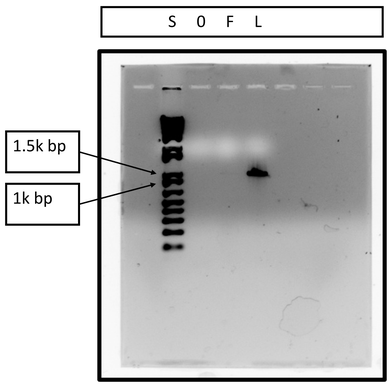


Figure 3.
Gel electrophoresis results showing the *SOS4* gene cloned from the cDNA of flowers and leaves of *A. thaliana*. Each lane is labeled with its contents. Key: S = Standard, 0 = Negative Control, F = Flower Sample, L = Leaf Sample. The Flower DNA band is between 1500 and 1000 bp in length.

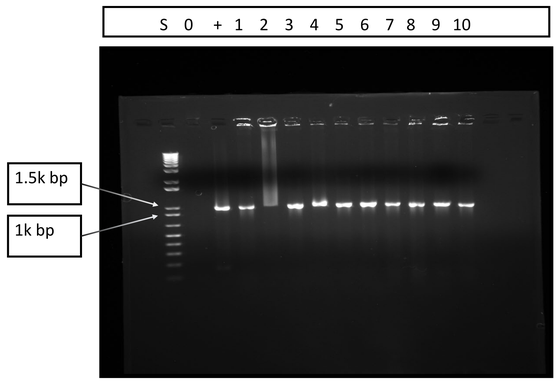
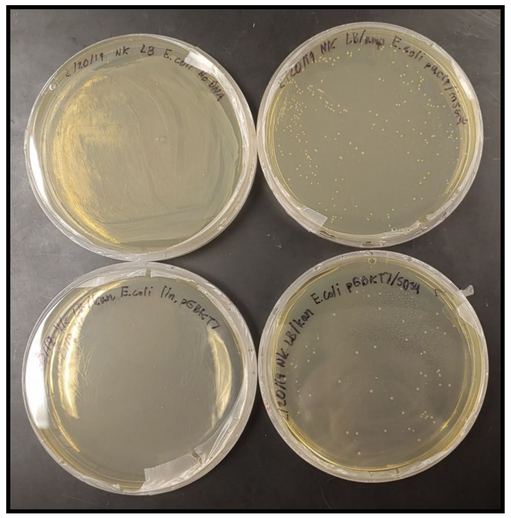


Figure 5.
Gel electrophoresis results showing the presence of *SOS4* DNA in transformed *E. coli*. Each lane is labeled with its contents. Key: S = Standard, 0 = Negative Control, + = Positive Control, # 1 – 10 = Experimental Samples. The DNA bands resulting from the experimental samples are between 1500 and 1000 bp in length.

Transformation Control
E. coli grown on LB with no additional DNA added.

Negative Control
E. coli grown on LB and kanamycin with linearized pGBKT7 vector added.



Positive Control
E. coli grown on LB and ampicillin with pUC19 insert added.

Experimental Plate
E. coli grown on LB and kanamycin with pGBKT7/*SOS4* added.

Figure 4.
Transformed *E. coli* colonies. The experimental plate contains colonies with the pGBKT7/*SOS4* construct.

Conclusion

- The *SOS4* gene from *A. thaliana* was cloned into PGBKT7.
- *E. coli* was transformed using pGBKT7/*SOS4* construct.
- Colony PCR of transformed *E.coli* colonies confirmed the presence of *SOS4*.
- *SOS4* positive colonies were grown in liquid cultures.
- The pGBKT7/*SOS4* construct was extracted from transformed *E. coli* cells.
- DNA was quantified and sequenced.

References

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Acknowledgements

- IUS Research Start Up Funds
- IUS McCullough Fund
- IUS Grant-in-aid
- IUS Large Grant